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PLASTICITY OF BIOLOGIC FORMS OF PUCCINIA GRAMINIS

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INTRODUCTION

Ever since the discovery of biologic forms of *Puccinia graminis* Pers. by Eriksson (*5*)[3] there has been much speculation as to the degree of fixity of these forms. Eriksson (*6, p. 657*) expresses the opinion that, on account of host and climatic influences, the forms may gradually change. His conception of a biologic form is that it is the result of an adaptational tendency. Magnus (*13, p. 366*) seemed to be of the opinion that biologic forms are the result of association with the particular host plants which they attack. Dietel (*4*) and others held essentially similar views. In general these assumptions have been considered reasonable.

Ward (*22, 23*), as a result of exhaustive investigation of the brown rust of bromes *Puccinia dispersa* Erikss., concluded that biologic forms of this rust could be changed parasitically by association with the proper host plants. He found that, whereas it was often impossible to transfer the rust directly from one species of Bromus to another species, this result could sometimes be accomplished by infecting a third species on which the rust acquired the capability of infecting the normally immune species. Such species he designated bridging species. His opinion appears to have been that taxonomic relationships among the species of Bromus were the determining factor. If, for example, the rust on a given species, A, could not be transferred directly to another species, C, it might be transferred to B, intermediate taxonomically between A and C. The species B then changed the rust sufficiently to enable it to infect C. Having once established itself on C, it was thenceforth capable of

[1] On leave.

[2] Published, with the approval of the Director, as Paper 127 of the Journal series of the Minnesota Agricultural Experiment Station.

[3] Reference is made by number (italic) to "Literature cited," p. 250-251.

infecting it easily. Freeman (*8*) did similar work and came to the same conclusions. No attempt has yet been made to repeat the work of these investigators, but the writers have observed that some species of Bromus are hosts for most biologic forms of *P. graminis*. *Bromus tectorum*, for instance, can be infected by all the common forms of *P. graminis* in the United States. It is possible that the conditions of experimentation on which the idea of bridging is based were not rigid enough to exclude all possibility of working with a mixture of biologic forms. On the other hand, it is quite possible that the brown rust of bromes with which Ward and Freeman worked was an unstable, easily changed form.

Salmon (*16*) added considerable evidence to the concept of the efficacy of bridging hosts in widening the host range of biologic forms by his experiments with *Erysiphe graminis* DC. on various species of Bromus.

Freeman and Johnson (*9*) applied the principle of bridging hosts to *P. graminis*. They state (*p. 20*) that—

> The barley stem rust enjoys the widest range of any of the biologic forms of the cereal rusts. On the other hand, a transfer of any of the other stem rusts to barley widens the range of that rust. We have here, then, a decided reaction of host upon parasite, enabling the latter to adapt itself to hosts not ordinarily congenial; for instance, W——>B——>O.

Johnson (*11, p. 10*) obtained similar results with timothy rust. He states—

> A small number of experiments to test whether or not the timothy rust can be transferred by means of bridging hosts to various cereals which are not successfully infected directly from timothy were tried, and it was found that by using *Avena sativa* as a bridging host the rust easily transferred to *Hordeum vulgare* (4 times in 10 trials); and by using *Festuca elatior* it transferred to *Hordeum vulgare* (twice in 10 trials) and to *Triticum vulgare* (once in 10 trials); and by using *Dactylis glomerata* it transferred to *Triticum vulgare* (once in 5 trials). By the use of the bridging hosts the rust undoubtedly could be made to transfer to many grasses on which it will not grow when coming directly from timothy, but on which it might continue to grow after such a transfer. That this takes place to some extent in nature is very probable, and these trials, together with recent experiments of a similar nature on the rusts of grains, throw much light on the possible origin of many of the so-called "physiological species" of rust.

Pole Evans (7) stated that hybrid wheats could also act as bridging hosts, enabling *P. graminis* to infect the susceptible parent more vigorously and even to attack the highly resistant or almost immune parent. Biffen (*2*), however, obtained no evidence of such remarkable changes.

Arthur (*1, p. 227–228*) cited evidence to show that barberry (*Berberis* spp.) may also act as a bridging host, enabling "racial strains" of *Puccinia poculiformis* (Jacq.) Wettst. (=*P. graminis* Pers.) to increase their range of infection capabilities. Bolley and Pritchard (*3*) and others attributed to barberry a "reinvigorating function" for the rust, although not necessarily a bridging function.

On account of the weight of the above-cited evidence the role of bridging hosts in breaking down biologic-form specialization has been given fairly general credence.

But Eriksson (*5*), Jaczewski (*10*), Freeman and Johnson (*9*) and Stakman (*18*) could detect no clearly appreciable influence of barberry on the parasitism of biologic forms of stemrust. Stakman (*18*), Stakman and Jensen (*19*), and Stakman and Piemeisel (*20*), in rather limited experiments, could not duplicate the results of Freeman and Johnson with *P. graminis* nor those of Johnson with *P. graminis phleipratensis* (= *P. phleipratensis*). Stakman (*18*), however, obtained results indicating that possibly changing the host metabolism by the use of anesthetics and fertilizers might increase the parasitic capabilities of the rust slightly, thus giving some support to the work of previous investigators (*12, 14, 15, 17*) along similar lines.

On account of the undoubted theoretical and practical importance of the problem it seemed desirable to make extensive experiments with a number of forms of rust from different hosts from different regions. Work was therefore begun in the summer of 1914 and continued uninterruptedly since that time.

Although there was already considerable evidence on the question of the effect of barberry on the rusts, it seemed desirable to do still further work. An attempt was therefore made to determine the possible role of barberry as a bridging host and also to determine its possible effect as a reinvigorator of the rust.

Material was obtained from as many different sources as possible for the work with cereals and grasses as intermediaries or bridging hosts. While much of the work with so many different strains might appear to be superfluous, nevertheless there has been some idea that strains of the same biologic form might differ somewhat parasitically. Naturally, therefore, it would be desirable to get data on as many different strains as possible.

Most of the work was done with the *tritici* and *secalis* forms because they are the most important economically in the spring-wheat States and because theoretically it seems probable that bridging should take place with these two forms on account of the close similarity of the rusts to each other in many respects. It has already been pointed out by the writers (*21*) that the *tritici* and *secalis* forms have many hosts in common. Thus, barley, various species of Elymus, Hystrix, Hordeum, and Agropyron are about equally congenial hosts for both rust forms. It would seem that if the idea of bridging is well founded, and if the host plants actually do exert a distinct and permanent effect on the rusts, these common hosts ought to unify rust strains which are grown on them.

Barley (*Hordeum* spp.) was used more than any of the other forms in attempted bridging on account of the fact that Freeman and Johnson

(9) found that it exerted such a pronounced effect on the rust, enabling any biologic form to increase its host range. The grasses were also used to a considerable extent, because both biologic forms of the rusts mentioned are so often found associated on them in the field. If rust changes rapidly, therefore, as a result of host influence, it would seem that the wheat stemrust and rye stemrust, growing on any one of these hosts for a number of urediniospore generations in the field, ought gradually to acquire the same parasitic capabilities.

The Agropyrons are especially interesting because the *tritici* form is able to attack some of them virulently (*A. tenerum* and *A. smithii*), while it can attack others, such as *Agropyron repens*, weakly or not at all. Here, then, there should be an opportunity to test the theory that taxonomic relationship determines the ability of the rust to pass from one host plant to another. If the stemrust of wheat (*Triticum* spp.) can not be transferred directly to *A. repens*, but can be developed normally on *A. tenerum* which is obviously more closely related to *A. repens* than to wheat, theoretically the rust should be able to pass from wheat to *A. repens* after it had first been transferred to *A. tenerum* or some other species of Agropyron closely related to *A. repens*.

Another problem is presented when a biologic form can attack a host plant weakly. If the theory of bridging hosts is a fact, certainly the rust must be easily changed by the host plants. Assuming, then, that individual plants of a given species vary in their susceptibility to the rust, it ought to be possible to increase the virulence of the rust on that particular host plant by successive inoculations with spores from the most vigorous uredinia. Or it ought even to be possible to increase the virulence of the rust by constant association with the uncongenial host. Rye is especially favorable for study in this respect. Individual rye plants vary very greatly in their susceptibility to *P. graminis tritici*. Some are entirely immune, others are almost immune, others are moderately susceptible, and still others are quite susceptible. Experiments were made to determine whether the rust from the susceptible plants when transferred to other rye plants could attack rye with greater virulence; also, whether it was possible to increase the virulence by simply transferring for a number of successive generations to rye. Similar conditions obtain when barley is inoculated with the *phleipratensis*, *avenae*, and *agrostis* strains, and experiments were made with other forms also.

As indicated in the historical summary, there seemed to be some evidence that changing the metabolism of the host might materially affect the parasitic capabilities of the rust. The writers therefore undertook further experiments along this line. The results of these experiments will be given in a separate paper not yet published.

If the biologic forms of *P. graminis* are easily changed, it seems reasonable to suppose, as previous experiments seem to have demonstrated

in a preliminary way, that this change brought about by a host plant should be manifested, not only in the parasitic capabilities of the rust, but also in the morphology. Extensive work was therefore done on this phase of the problem, and the results will be presented in a separate paper.

EXPERIMENTAL METHODS

The methods used in inoculating and incubating the plants were similar to those described by Stakman and Piemeisel (*21, p. 431–432*). On account of the fact that conclusions would be difficult to draw unless the experimental methods excluded to the greatest extent possible accidental infection, the utmost precautions were taken to prevent accidental infection. This is rather difficult when working under the conditions necessary in such an investigation. Unfortunately the pure-culture methods of bacteriology and mycology can not be successfully employed for rusts. However, it is possible to reduce the number of accidental infections to a very small minimum.

The seedling plants used in the experiments were grown under cages made of two layers of fine-mesh muslin separated by a dead-air space about an inch wide. Immediately after inoculation they were placed under bell jars, and as soon as the incubation period was over they were again placed under cages similar to those under which the seedling plants were grown.

Every precaution was taken to prevent infection from the outside by air-borne spores and to destroy all infected material as soon as possible. When accidental infection did take place, it could practically always be determined with certainty, although in a few cases it was not possible to do this. When, for instance, a leaf of wheat, as very rarely indeed happened, developed a normal rust uredinium as a result of inoculations with *P. graminis secalis* from rye, and when the spores in the uredinium morphologically and parasitically were in every way typical of *P. graminis tritici* spores, it seemed more reasonable to assume that accidental infection had taken place than to assume that bridging had occurred or that the rust had mutated. This conservatism in interpreting results seemed to be especially necessary when such a tremendous preponderance of evidence accumulated showing that bridging and mutations did not occur.

It was soon found also that the greatest precaution was necessary in being absolutely certain that the biologic form in use in a given experiment was absolutely pure before the results could be clearly interpreted. In making inoculations from barley and rye and many grasses from the field, both the *tritici* and *secalis* forms develop quite often. It is possible to inoculate all of the cereals and to obtain results which seem to show that only one biologic form is present, then to inoculate one of the common hosts for both forms, and then to find that a very small amount

of the other biologic form had been present on the original material, but was only given an opportunity to develop as a result of one or more transfers to the common host.

In experiments covering only a short period of time and with a small number of forms it might easily be concluded that bridging had occurred. The danger of drawing such conclusions, however, is clearly shown in diagrams 1 to 4, inclusive. Many cases of apparent bridging were seen, but in every case, with possibly one or two exceptions, it could be shown conclusively that this was due to the fact that more than one biologic form had been used.

The cereals used were the following, unless otherwise specified: Oats, Improved Ligowa (Minnesota 281); barley, Manchuria (Minnesota 105); wheat, Haynes Bluestem (Minnesota 169); rye, Swedish (Minnesota 2). Most of the grass seeds used were obtained by or through the Minnesota Seed Laboratory.

KEY TO TABLES I AND II AND DIAGRAMS 1 TO 10

The results of experiments to determine the effect of bridging hosts on the parasitism of the rust forms are given in Tables I and II and diagrams 1 to 10. The results of inoculations are usually given in the form of a fraction, the denominator indicating the total number of leaves inoculated and the numerator the number which developed uredinia. Whenever the presence of flecks is indicated, the number of leaves flecked is given after the semicolon following the fraction. Two types of diagrams are used—complete and condensed. Wheat, oats, barley, and rye are designated as W, O, B, and R, respectively. The names of the grasses are either written out in full or the key to the abbreviations is given in connection with each diagram. The sequence of transfers is indicated by dashes, proceeding from left to right. All of the plants indicated in the same vertical line after a dash were inoculated with the rust from the host immediately preceding the dash. Not all of the inoculations are indicated, since this would require altogether too much space. The essential ones, however, are indicated in the so-called complete diagrams, while summaries only are given in the condensed diagrams.

In the condensed diagrams the small number immediately following the symbol for cereal hosts or the names of the grass hosts indicates the number of urediniospore generations on that particular host. The fraction in parentheses indicates the result of inoculations which have been made during that period. The denominator gives the total number of plants inoculated and the numerator the number which became infected. The number of sets of inoculations is not indicated. Usually, although not always, the immune or highly resistant host was inoculated each urediniospore generation. For instance, "R_2—B_4—*Elymus canadensis*$_5$" indicates that two successive transfers had been made to rye followed by four successive transfers to barley and five to *Elymus canadensis*. These are spoken of as urediniospore generations. The transfers were usually made at intervals of approximately two weeks, so that each urediniospore generation represents about that length of time. "R_2 (wheat $\frac{0}{50}$)—B_4 (wheat $\frac{0}{25}$)—*Elymus canadensis*$_5$ (wheat $\frac{0}{35}$)" means that during the two generations on rye 50 leaves of wheat were inoculated, none of which became infected. One of the inoculations was probably made the first generation and the other the second generation. The rust was then transferred to barley and kept there for four

generations, during which 25 leaves of wheat were inoculated, none of which became infected. Usually this includes trials from most of the generations. The rust was then kept on *Elymus canadensis* for five generations, during which 35 leaves of wheat were inoculated without producing infection. "R_2—B_4 (wheat $\frac{0}{30}$)" would indicate that from the rust on barley 30 leaves of wheat had been inoculated, but none had been inoculated directly from rye.

When "strains" of rust are spoken of, the word is used in the sense of a biologic form with a certain history without any imputation that it is in any way different from a normal form.

The terms "intermediary host" and "bridging host" or "bridging species" are used in the theoretical sense—that is, hosts which apparently ought to cause bridging are spoken of as bridging hosts without any suggestion that they actually do cause bridging. The term "common host" is sometimes used. This means a plant which is a host for the biologic forms under discussion at that particular time.

EXPERIMENTS WITH BARBERRY AS A BRIDGING HOST

To test the ability of barberry to break down biologic forms and its capacity for "reinvigorating" the rust parasite, inoculation experiments were carried on during four æcial seasons. The teliospores were obtained from wheat, club wheat, oats and a few grasses. The common barberry (*Berberis vulgaris*) was used as the æcial host, and the æciospores produced were used for inoculating wheat, oats, barley, and rye. Four biologic forms of *P. graminis*—viz, *tritici*, *tritici-compacti*, *avenae*, and *secalis* were studied and the results obtained are given in Table I.

TABLE I.—*Results of inoculating cereals with æciospores of biologic forms of Puccinia graminis*

No.	Date.	Original sources of rust.	Biologic form.	Result.			
				W.	O.	B.	R.
1	May 25, 1914	Wheat	*tritici*	$\frac{4}{24}$	$\frac{0}{20}$	$\frac{3}{24}$	$\frac{0}{30}$
2	June 3, 1914	....do	....do	$\frac{13}{16}$	$\frac{0}{18}$	$\frac{1}{18}$	$\frac{2}{14}$
3	June 9, 1914	....do	....do	$\frac{9}{20}$	$\frac{0}{19}$	$\frac{11}{22}$	$\frac{1}{20}$
4	June 26, 1914	....do	....do	$\frac{6}{20}$	$\frac{0}{30}$	......	$\frac{1}{13}$
5	May 8, 1915	....do	....do	$\frac{5}{10}$	$\frac{0}{14}$	$\frac{10}{14}$	$\frac{0}{12}$
6	May 23, 1915	....do	....do	$\frac{15}{34}$	$\frac{0}{35}$	$\frac{2}{28}$	$\frac{1}{14}$
7	June —, 1916	....do	....do	$\frac{9}{20}$	$\frac{0}{19}$	$\frac{11}{22}$	$\frac{1}{20}$
8	Apr. 11, 1917	Club wheat	*tritici-compacti.*	[a] $\frac{2}{33}$	$\frac{0}{13}$	$\frac{12}{16}$	$\frac{1}{10}$
9	June 8, 1916	Oats	*avenae*	......	$\frac{12}{14}$	[b] $\frac{1}{29}$	......

[a] *Puccinia graminis tritici-compacti*; very weak infection on wheat; normal infection ($\frac{8}{14}$) on club wheat.
[b] Minute uredinium.

TABLE I.—*Results of inoculating cereals with æciospores of biologic forms of Puccinia graminis*—Continued

No.	Date.	Original sources of rust.	Biologic form.	Result.			
				W.	O.	B.	R.
10	June 14, 1916	*Sporobolus cryptandrus.*	*secalis*	0/20	0/5	3/5	8/11
11	May 25, 1914	*Agropyron repens.*	....do	0/17	0/20	4/19	3/20
12	June 3, 1914	....do	....do	0/18	0/19	5/16	2/15
13	June 9, 1914	....do	....do	1/22	0/21	0/15	8/22
14	....	....do	....do	0/10	0/10	7/9	7/8
15	May 31, 1917	....do	....do	0/20	2/21	17/18	......
16	July 2, 1917	*Agrostis stolonifera.*	....(?)	9/41	4/27	......	14/26

Barberry probably does not increase the host range of biologic forms commonly. If it did, æciospores collected in the field ought to infect cereals and grasses more indiscriminately than they do. On the other hand, the biologic specialization in the æcial stage is apparently the same as that in the uredinial stage. The percentage of infected leaves was often low, but this is often true when inoculations are made with æciospores. All of the results recorded in Table I, except No. 16, might equally well have been the result of inoculations with urediniospores, except that a larger percentage of inoculated leaves would have been infected.

The results shown in No. 16 can not be explained, unless accidental infection took place. The grass was collected in the fall and kept until the next July. The barberry had been kept in the greenhouse under a double muslin cage until inoculated and was again covered immediately after removal from the incubating chamber. Three biologic forms developed on the barberry; how they got there can not be stated with certainty.

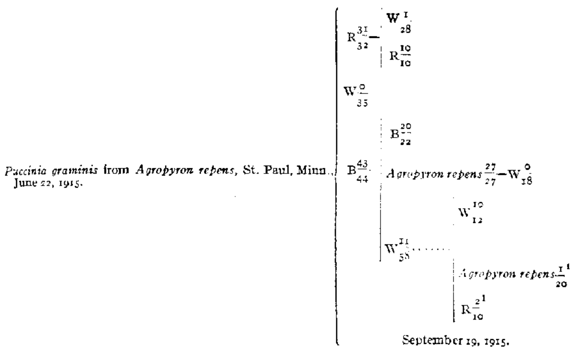
EXPERIMENTS WITH CEREALS AND GRASSES AS BRIDGING HOSTS

Many hosts equally susceptible to *P. graminis secalis* and *P. graminis tritici* were used as bridging hosts in attempts to change the parasitism of the two forms. Barley, various species of Elymus, Agropyron, Hordeum, and Bromus were used most. *Bromus tectorum* was used as

a possible bridging host for *P. graminis avenae*. Special care was taken to isolate the biologic forms whenever they were mixed, in order to avoid conflicting results and erroneous conclusions. The results of this phase of the work which extended for a period of over three years are given in diagrams 1 to 10 and in Table II.

It might have been concluded from the results shown in diagram 1 that barley acted as a bridge between *Agropyron repens* and wheat. It is shown quite clearly, however, by subsequent inoculations that it did not. The rust on the original quack-grass no doubt was mostly of the *secalis* form. It had probably been contaminated slightly in the field with some of the *tritici* form. Since there was only a small amount of the latter form, none developed on the wheat in the original inoculations. A very small amount developed on rye and more developed on barley. Therefore, when inoculations were made with the rust from barley, both the *tritici* and *secalis* forms were present, but they were separated in the third set of inoculations. A more complicated condition is shown in diagram 2.

DIAGRAM 1.—Results of inoculations with *Puccinia graminis* from *Agropyron repens* showing apparent bridging before biologic forms were isolated.



[1] Small uredinia; *P. graminis tritici*.

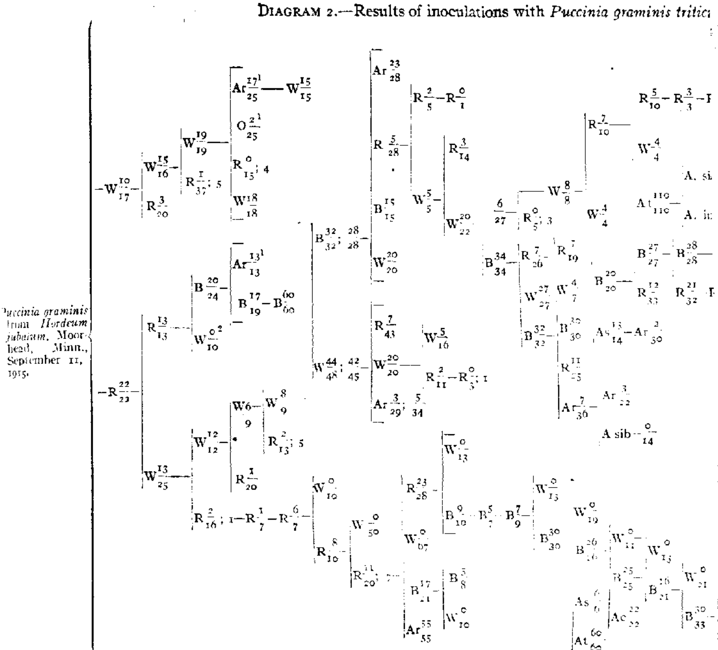
In diagram 2 both wheat and rye became infected when inoculated with the rust from *Hordeum jubatum*. The rust developed on wheat proved to be a pure strain of *tritici*. That developed on rye, however, consisted of both the *tritici* and *secalis* forms. Very clearly the original rust on *Hordeum jubatum* was a mixture of the *tritici* and *secalis* forms. The *tritici* form was isolated in pure form by transferring to wheat on which the *secalis* form did not develop. But the first generation of the rust on rye was still mixed, since *tritici* develops weakly on rye. Apparently the second generation of the rust on rye was pure *secalis*, since it did not develop on wheat, the leaves of which, however, died young. But, after having passed four generations on barley, the rust infected wheat normally and infected rye only weakly. This clearly looked like bridging. The more probable explanation, however, is that both the *secalis* and *tritici* forms again developed the second generation on rye. Since the wheat plants died young, the negative results recorded are not significant.

In the subsequent inoculations both *secalis* and *tritici* probably developed on barley, since a high percentage of inoculated leaves of *Agropyron repens* became infected. The *secalis* form, however, was eventually lost. This may have happened in two ways. The *tritici* form may have developed more rapidly than the *secalis*; or material from the leaves infected with *secalis* may not have been used in making inoculations It is quite probable that if rye had been inoculated earlier both forms would have been isolated.

From the R_1—W_1 material both biologic forms were isolated. This was puzzling at first, because wheat is not a host for the *secalis* form. The only plausible explanation seemed to be that spores of both biologic forms were placed on the wheat during inoculation and not all germinated in the moist chamber. A few viable *secalis* spores therefore remained on the wheat, and when these were transferred to rye, they germinated, causing infection. In order to ascertain whether this was possible, wheat was inoculated with the *secalis* form, kept in a moist chamber for 48 hours, and then kept on a greenhouse bench for about 10 days. No rust developed, but the inoculum was scraped from the leaves and used to inoculate rye plants. Infection resulted on a relatively large number of leaves, showing that the theoretical explanation advanced above was probably correct. These facts show that extreme caution is necessary in drawing conclusions when dealing with mixed forms of rust.

The most convincing proof that the rust forms, after isolation in pure form, could not be changed by host influences is furnished by the subsequent history given in diagram 2. The *tritici* form did not acquire new parasitic ability on account of its association with barley; nor did it increase in virulence on rye as a result of successive transfers. It will be noted that repeated unsuccessful attempts were made to develop a

DIAGRAM 2.—Results of inoculations with *Puccinia graminis tritici*



78776°—18. (To face page 230.)

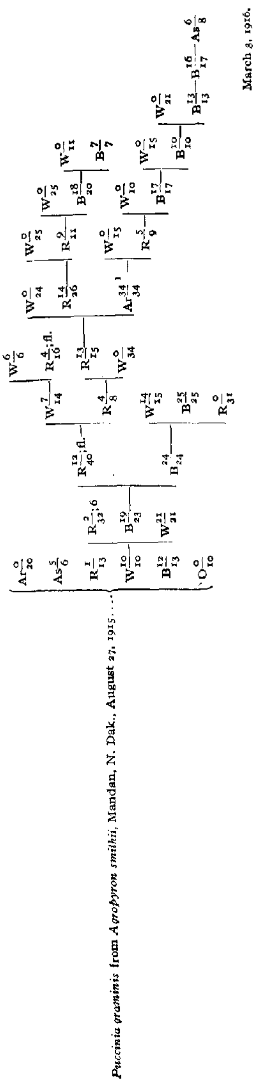


DIAGRAM 3.—Results of inoculations with *Puccinia graminis* from *Agropyron smithii*, Mandan, N. Dak.

[1] Small uredinia.

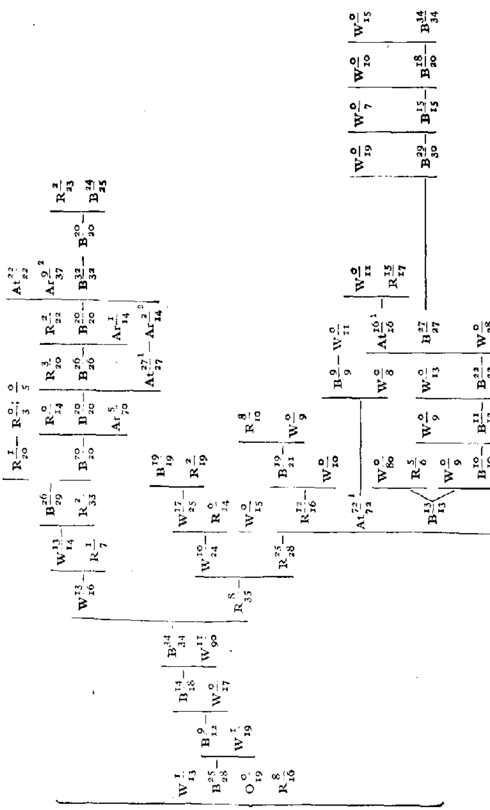
Ar= *Agropyron repens.*
As= *Agropyron smithii.*

strain capable of attacking rye normally. Diagram 2 shows also that the susceptible species of Agropyron used did not act as bridges to normally immune species of this genus. The rust was kept on barley for over a year, but it proved to be entirely stable and was therefore discarded. The *secalis* form likewise remained fixed after it was isolated and attacked its regular hosts vigorously, but could not be transferred to wheat or other normally immune hosts, a large number of which were inoculated, but the results of which are not given in the diagram for want of space.

Erroneous conclusions might easily have been drawn from the results shown in diagram 3 if the inoculations had not been extensive. Here, again, the original rust was a mixture consisting mainly of the *tritici* form but including also a small amount of *secalis*. Viable spores of *secalis* were again carried through the moist chamber, and these infected barley, thus accounting for the later events. The *secalis* form was kept for about five months, but did not change.

DIAGRAM 4.—Effect of intermediary hosts on *Puccinia graminis secalis* and *P. graminis tritici* from *Agropyron cristatum*.

Puccinia graminis secalis and *P. graminis tritici* from *Agropyron cristatum*, St. Paul, Minn., September 10, 1915.



April 18, 1916.

[1] Heavy infection.
[2] Weak infection.

At=*Agropyron tenerum*.
Ar=*A. repens*.

The original rust was mixed here (diagram 4) also, and only after six successive sets of inoculations had been made were the *tritici* and *secalis* forms finally separated. Neither barley nor *Agropyron tenerum* acted as bridging hosts for the *tritici* form. The *secalis* form likewise remained fixed and did not acquire the ability to attack wheat after growing on either barley or *Agropyron tenerum.*

DIAGRAM 5 (condensed).—*Results of inoculations made with Puccinia graminis secalis from Hystrix patula.*

Puccinia graminis secalis from *Hystrix patula*, St. Paul, Minn., September 2, 1915.

$R_2-B_4-\left(W\frac{0}{17}\right)-R_2-\left(W\frac{0}{34}\right)$

$-B_2-\left(W\frac{0}{10}\right)-Ar_1-B_1-Ev_3-$

$At_4-B_{22}-\left(W\frac{0}{104}\right)$

$At_9-\left(W\frac{0}{84}\right)$

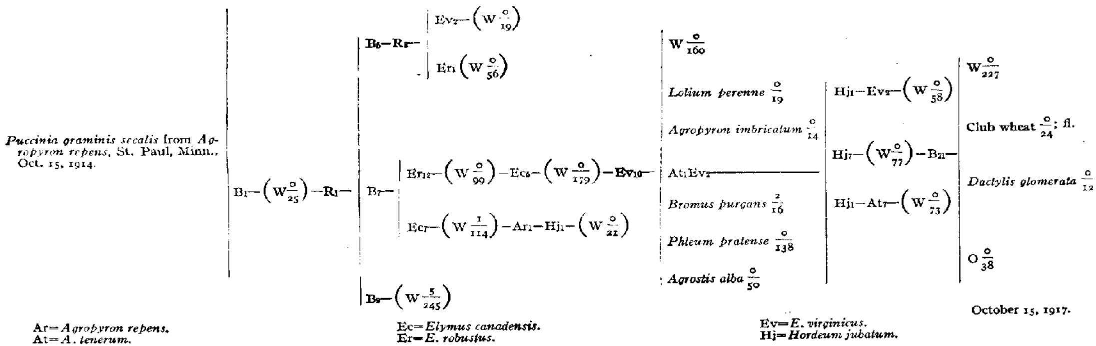
$At_4-Hj_2-\left(W\frac{0}{56}\right)$

September 17, 1917.

Ar=*Agropyron repens.*
At=*Agropyron tenerum.*
Hj=*Hordeum jubatum.*
Ev=*Elymus virginicus.*

The strain of *P. graminis secalis* from *Hystrix patula* was somewhat different from normal *secalis* strains (diagram 5). It was not as virulent on barley and rye, and the spores were somewhat smaller. Attempts were made to induce the rust to attack wheat by growing it on barley, *Elymus virginicus*, *Agropyron tenerum*, and *Hordeum jubatum.* None, however, acted as a bridge. The rust was kept for over two years, during which 18 sets of inoculations were made on wheat; but none of the 305 inoculated leaves became infected, except in one case, which was quite evidently an accidental infection with *P. graminis tritici.* However, the rust did not act normally and may have been a different biologic form. Some of the results were difficult to explain, and more work will probably be done with it.

DIAGRAM 6 (condensed).—Results of inoculations with *Puccinia graminis secalis* from *Agropyron repens* after various intermediary hosts.



This strain of *P. graminis secalis* was obtained (diagram 6) from *Agropyron repens* at St. Paul, Minn., on October 15, 1914, and was kept until October 15, 1917. It was confined to intermediary hosts for three years, during which time about 60 different sets of inoculations were made on wheat. About 1,800 leaves were inoculated, some of which are not recorded in diagram 6, and only six became infected. There is strong reason to suspect that these were accidentally infected with the *tritici* form, all occurring during the earlier period of work. Barley, *Elymus robustus*, *E. canadensis*, *E. virginicus*, *Agropyron tenerum*, and *Hordeum jubatum* were all used as intermediary or bridging hosts, but none of them enabled the rust to transfer to wheat, which remained practically immune throughout the entire period. Flecks very seldom developed. Since these hosts, which ought to act as bridging hosts, if any hosts act in this manner, did not enable the rust to transfer to wheat after three years of continuous culture, it seems quite improbable that such a change would take place quickly in nature.

In Table II a number of miscellaneous experiments are included. The most conclusive is No. 1, in which the *secalis* strain was kept continuously on barley for 16 successive urediniospore generations, covering a period of eight months. During this time 11 sets of inoculations were made on wheat, but none of the 264 leaves inoculated became infected, except one, which was very clearly shown to be accidental. In all of the other trials with the other strains of rust no infection whatever resulted on the wheat. It is quite clear, then, that under the conditions of these experiments neither barley nor *Elymus robustus* enabled the rust to bridge over to the normally immune wheat. This result could hardly be expected, because if such changes did take place in nature the biologic forms isolated from these hosts could not be as uniform as they are.

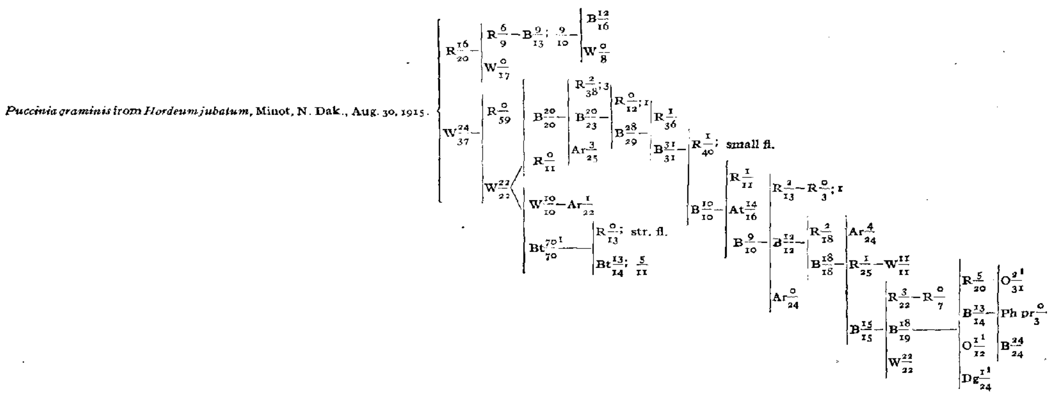
The last two experiments (No. 6 and 7), on the other hand, show the inability of barley to serve as a bridging host for the *tritici* strain, or to induce this rust to infect rye more vigorously than it ordinarily does.

TABLE II.—*Results of attempts to transfer Puccinia graminis secalis to wheat and P. graminis tritici to rye by using barley and Elymus robustus as intermediary hosts*

No.	Original host.	Place of collection.	Intermediary host.	Number of generations on intermediary host.	Time on intermediary host.	Number of trials.	Plant inoculated.	Number of leaves inoculated.	Number of leaves infected.
1	*Agropyron repens.*	Presque Isle, Me.	Barley	16	8 months	11	Wheat	264	[a] 0
2	Do	University Farm, Minn.	do	1	2 weeks	1	do	59	0
3	Do	do	do	1	do	1	do	33	0
4	Do	Groveland, Minn.	do	1	do	1	do	30	0
5	Do	do	*Elymus robustus.*	1	do	1	do	11	0
6	*Hordeum jubatum.*	Berwick, N. Dak.	Barley	1	2½ weeks	1	Rye	42	0
7	Do	Lisbon, N. Dak.	do	4	1½ months	4	do	90	[b] 0

[a] One leaf accidentally infected with *P. graminis tritici*.
[b] Four flecks in 2 out of 4 trials.

DIAGRAM 7.—Results of attempts to increase the infection capabilities of *Puccinia graminis* from *Hordeum jubatum*, Minot, N. Dak.



Ph pr= *Phleum pratense*.
At= *Agropyron tenerum*.

Bt= *Bromus tectorum*.
Ar= *Agropyron repens*.

Dg= *Dactylis glomerata*.

[1] Minute uredinia.

The rust from the *Hordeum jubatum* in this series (diagram 7) consisted originally of both the *tritici* and *secalis* forms. However, they were easily separated and remained fixed after separation. The *secalis* strain was kept only for a short time, since it proved to be an ordinary strain which failed to attack wheat either when transferred directly from rye or after having been transferred to barley. The *tritici* strain, however, was kept for some time in the expectation that it might be induced to attack rye more vigorously. Barley was used almost exclusively as a bridging host, but it is perfectly clear that it did not enable the rust to attack rye any more vigorously than the rust taken directly from wheat. Neither did it acquire the power to attack *Agropyron repens*, *Dactylis glomerata*, oats, or *Phleum pratense* any more readily than the rust taken directly from the wheat. It should be remembered that rye and *Agropyron repens* can often be attacked weakly by the *tritici* form, while oats and *Dactylis glomerata* are very rarely attacked, and timothy has so far proved entirely immune. One attempt was made to use *Bromus tectorum* as a bridging host, but it did not enable the rust to transfer to rye. In fact, no uredinia were developed, only a few strong flecks.

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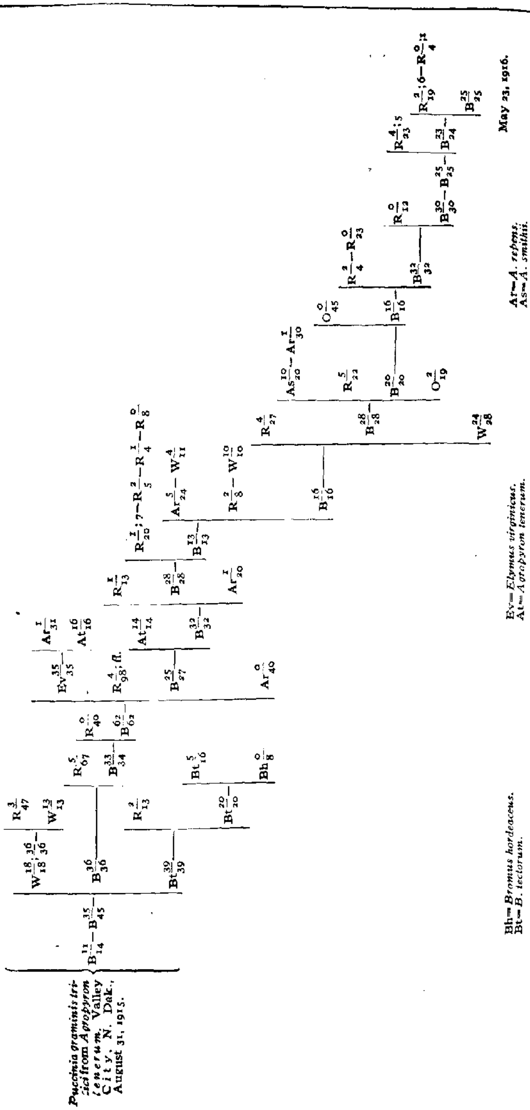
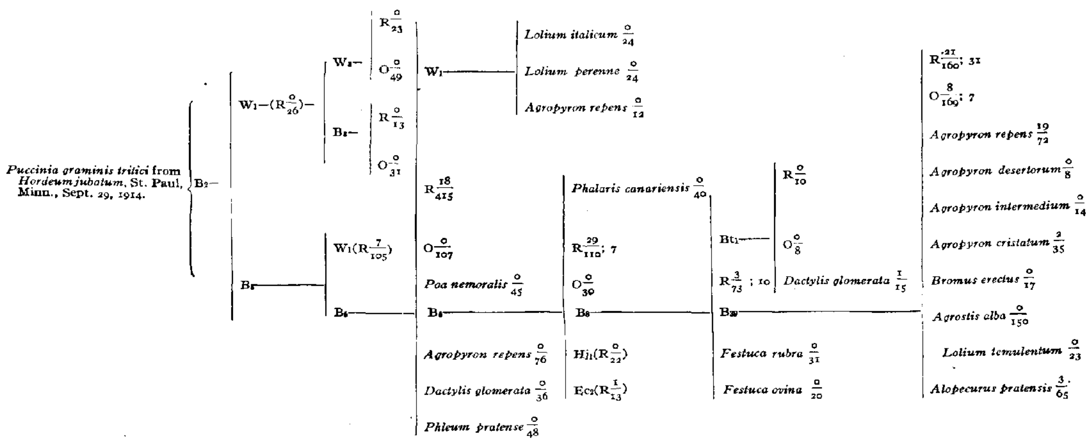
DIAGRAM 8.—Results of successive transfers of *Puccinia graminis tritici* from *Agropyron tenerum* to barley and other hosts.



The strain of *tritici* used for the inoculations represented in diagram 8 was originally obtained from *Agropyron tenerum* at Valley City, N. Dak. It was one of the most vigorous *tritici* strains which has been obtained, but it was incapable of attacking rye, *Agropyron repens*, or oats with any degree of success, after having been kept on barley for a number of generations. The rust transferred readily to *Bromus tectorum* and was transferred from this host again to *Bromus tectorum* and *B. hordeaceus*, but it acquired no new power as a result of its sojourn on *B. tectorum*. *Elymus virginicus* was used in an attempt to get the rust to transfer to *Agropyron repens* but without success, only one small uredinium developing on one of the 31 inoculated leaves. *Agropyron smithii*, which is a congenial host, was inoculated and transfers then made to *Agropyron repens* in order to further test the taxonomic relationship theory. The results, however, were disappointing. Successive transfers were made to rye with urediniospores from a large uredinium which developed in one set of inoculations, but the rust died after three successive inoculations had been made.

DIAGRAM 9 (condensed).—Results of inoculations with *Puccinia graminis tritici* from *Hordeum jubatum* after various intermediary hosts.

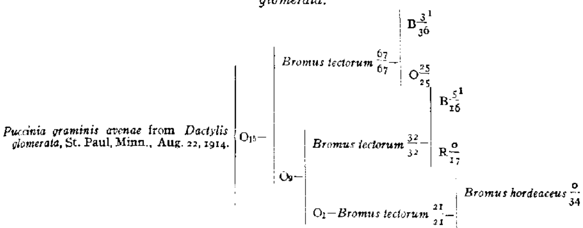


Bt=*Bromus tectorum*. Ec=*Elymus canadensis*. Hj=*Hordeum jubatum*.

June 15, 1917.

The *tritici* strain used in this series of inoculations (diagram 9) was isolated originally from *Hordeum jubatum* at St. Paul, Minn., on September 29, 1914, and was kept in uredinial culture until June 15, 1917. Barley, *Elymus canadensis*, *Bromus tectorum*, and *Hordeum jubatum* were all used as intermediary hosts, but none of them enabled the rust to do anything which it could not do at the beginning of the experiment. The results of inoculations with the rust developed on *Bromus tectorum* are particularly interesting, since this grass is a host for the six common biologic forms of *P. graminis* in the United States. Theoretically when the *tritici* form was transferred to it, since it is a congenial host also for the *secalis* and *avenae* strains, it should have transferred to rye and oats. However, it did not.

DIAGRAM 10.—Results of inoculations with *Puccinia graminis avenae* from *Dactylis glomerata*.



[1] *Minute uredinia.*

The *avenae* strain represented in diagram 10 was isolated originally from *Dactylis glomerata* at St. Paul, Minn. It has been kept on oats for about three years. A large number of inoculations were made on various hosts during that time, but it has never performed differently from the rust on *Dactylis glomerata*. *Bromus tectorum* was used as a bridge in attempts to induce the rust to pass more readily to barley and rye, since it is a host for both the *tritici* and *secalis* forms as well as for the *avenae* form, but it is clear that it did not do this. The uredinia developed on barley were minute, and the percentage of infection was no greater than that which is obtained from transfers made directly from oats or any other host for the *avenae* form, a large number of which have been tried.

EXPERIMENTS ON THE ADAPTION OF BIOLOGIC FORMS TO SEMI-CONGENIAL HOSTS

In order to determine whether or not biologic forms are capable of adapting themselves to semicongenial hosts through constant association, many attempts were made to increase the virulence of the rusts on these hosts by means of continuous, successive transfers. The biologic forms of *P. graminis tritici*, *P. graminis avenae*, *P. graminis phleipratensis*, and *P. graminis agrostis* were studied and the results are given in Tables III to VI, inclusive.

TABLE III.—*Results of successive transfers of Puccinia graminis tritici from various sources to rye*

Original host.	Place of collection.	Previous history of rust.	Result.
Agropyron caninum	Emerson, Manitoba	W_1	$R\frac{3}{38};\ 10-R\frac{4}{9};\ 2-R\frac{1}{13};\ 2-R\frac{0}{2}$
Agropyron cristatum	St. Paul, Minn	$B_4-W_2-B_8$	$R\frac{3}{20}-R\frac{1}{7}-R\frac{2}{5}-R\frac{0}{2};\ \frac{1}{4}-R\frac{0}{2}$
Agropyron tenerum	Valley City, N. Dak	B_3	$R\frac{1}{20};\ 7-R\frac{2}{5}-R\frac{1}{4}-R\frac{0}{8}$
Do	do	B_{16}	$R\frac{4}{23};\ 5-R\frac{2}{10};\ 5-R\frac{1}{5};\ 2-R\frac{0}{3}$
Do	Emerson, Manitoba	W_1	$R\frac{4}{48};\ 13-R\frac{4}{13};\ 7-R\frac{0}{18};\ 11$
Do	Glasgow, Mont	W_1	$R\frac{5}{25};\ 6-R\frac{1}{27};\ 5-R\frac{0}{2}$
Do	do	W_1	$R\frac{5}{44};\ 4-R\frac{1}{7};\ 2-R\frac{0}{2}$
Do	Crookston, Minn	W_1	$R\frac{9}{30};11-R\frac{4}{51};\ 19-R\frac{1}{10};8-R\frac{1}{9}-R\frac{0}{1}$
Elymus macounii	Winnipeg, Manitoba	W_1	$R\frac{12}{46};37-R\frac{9}{43};28-R\frac{9}{20};11-R\frac{4}{17};4-R\frac{0}{3}$
Hordeum jubatum	Two Harbors, Minn	W_2	$R\frac{2}{20};\ 5-R\frac{1}{11};\ 8-R\frac{0}{3}$
Do	Grand Rapids, Minn	W_1	$R\frac{10}{39};\ 6-R\frac{8}{31};\ 6-R\frac{3}{31};\ 10-R\frac{0}{13};\ 3$
Do	Minot, N. Dak	W_1	$R\frac{1}{11};\ 1-R\frac{0}{2}$
Do	Cut Bank, Mont	W_1	$R\frac{4}{34};\ 11-R\frac{2}{30};\ 1-R\frac{2}{5}-R\frac{1}{4};1-R\frac{0}{5}$
Do	Williston, N. Dak	W_1	$R\frac{2}{30};\ 4-R\frac{0}{2}$
Do	Emerson, Manitoba	W_1	$R\frac{6}{31};\ 14-R\frac{2}{19};\ 11-R\frac{0}{5}$
Do	Moorehead, Minn	$R_2-B_4-R_1-W_1$	$R\frac{3}{14}-R\frac{3}{11}-R\frac{1}{3}-R\frac{0}{1}$
Do	do	$R_2-B_4-R_1-W_2-B_1$	$R\frac{7}{26}-R\frac{7}{19}-R\frac{1}{6}-R\frac{1}{2}-R\frac{0}{2}$
Do	do	$R_2-B_4-R_1-W_2-B_4$	$R\frac{12}{33}-R\frac{12}{20}-R\frac{3}{11};4-R\frac{5}{24}-R\frac{1}{5};1-R\frac{0}{5}$
Do	do	$R_2-B_4-R_1-W_2-B_8$	$R\frac{1}{29};\ 9-R\frac{2}{7};\ 2-R\frac{3}{6};\ 3-R\frac{0}{2}$
Hordeum vulgare	St. Paul, Minn	B_8	$R\frac{9}{21};\ 9-R\frac{8}{22};\ 9-R\frac{2}{10};\ 4-R\frac{0}{3}$
Do	do	W_9	$R\frac{5}{28};\ 17-R\frac{6}{25};\ 12-R\frac{0}{2}$
Do	do	W_{10}	$R\frac{1}{13};\ 4-R\frac{0}{4}$
Do	do	W_x[1]	$R\frac{2}{7}-R\frac{0}{3}$

[1] x= Long-time association with host; number of urediniospore generations indefinite.

In Table III the results of a large number of attempts to increase the virulence of the *tritici* strain on rye by means of constant association with this host are given. It will be noticed that the rust was obtained from a number of different sources and from a number of different localities. The experiments covered a period of about three years. Whenever normal infection occurred on a rye plant the rust was transferred to other rye plants in the hope that it might prove to be a mutation or a tendency toward the differentiation of a strain of *tritici* capable of infecting rye normally. It will be seen, however, that this was not accomplished. The rust invariably gradually died out. Sometimes it seemed to increase in virulence as a result of successive transfers, often due to the abundance of infective material. At other times it apparently increased in virulence, owing merely to a change in experimental conditions. In several trials the rust had previously been on intermediary hosts for a number of generations, but this made no difference in the results. It so happened that some of the best results were obtained when the rust was taken directly from wheat. It was never possible to keep the rust on rye more than six generations. It simply became unthrifty and eventually failed to produce any uredinia, although excellent conditions for infection and subsequent development of rust were maintained.

TABLE IV.—*Results of attempts to increase the virulence of Puccinia graminis avenae by successive transfers to uncongenial hosts*

No.	Original host.	Place of collection.	Previous history of rust.	Result.
1	*Dactylis glomerata*....	St. Paul, Minn...	O_{29}......	$B\frac{3}{24}-B\frac{0}{3}$
2	*Panicularia pauciflora*	Whitefish, Mont..	O_1.......	$B\frac{12}{14}-B\frac{0}{24}$
3	Do.............	.....do..........	O_1-Dg_1	$Ph\ pr\frac{7}{35}-Ph\ pr\frac{0}{8}$
4	*Dactylis glomerata*....	St. Paul, Minn....	O_5......	$Bt\frac{10}{10}-Bt\frac{12}{14}-Bt\frac{5}{12}-Bt\frac{0}{4}$

Bt=*Bromus tectorum*. Dg=*Dactylis glomerata*. Ph pr=*Phleum pratense*.

The *avenae* strains used in the inoculations represented in Table IV were not able to develop increased virulence as a result of successive transfers on barley, *Phleum pratense*, or *Bromus tectorum*. The rust was kept on *B. tectorum* longer because this host is the most congenial of the three. However, it gradually died.

TABLE V.—*Results of successive transfers of Puccinia graminis phleipratensis to barley and oats*

No.	Original host.	Place of collection.	Previous history of rust.	Result.
1	*Festuca elatior.*	Sheridan, Wyo.	None....	$O\frac{7}{26}$; $4-O\frac{1}{18}-O\frac{1}{2}$
2	Do......	.....do.......	...do....	$B\frac{9}{25}$; $16-B\frac{0}{5}$; 5
3	Do......	Bellingham, Wash.	..do....	$O\frac{14}{31}-O\frac{16}{17}-O\frac{3}{13}-O\frac{1}{23}-O\frac{1}{9}-O\frac{0}{14}$
4	*Festuca pratensis.*	Pullman, Wash.	..do....	$B\frac{14}{20}-B\frac{9}{14}$; $1-B\frac{8}{8}-B\frac{0}{3}$; 1
5	*Phleum pratense.*	....do.......	...do....	$B\frac{7}{13}-B\frac{7}{12}$; $5-B\frac{1}{9}$; $4-B\frac{1}{3}-B\frac{1}{6}-B\frac{1}{1}-B\frac{0}{1}$
6	Do......	Ellensburg, Wash.	..do....	$B\frac{18}{31}-B\frac{0}{20}$; 2
7	Do......	Crawford, Nebr.	..do....	$B\frac{10}{44}-B\frac{3}{23}$; $4-B\frac{0}{4}$
8	*Dactylis glomerata.*	St. Paul, Minn.	Ph pr_6...	$O\frac{2}{13}-O\frac{2}{14}-O\frac{1}{8}-O\frac{0}{1}$
9	Do......	.....do.......	None.....	$O\frac{14}{53}-O\frac{10}{26}-O\frac{9}{20}-O\frac{2}{16}-O\frac{0}{2}$
10	Do......	.....do.......	...do.....	$O\frac{6}{12}-O\frac{3}{16}-O\frac{4}{7}-O\frac{8}{8}-O\frac{0}{5}$

Ph pr=*Phleum pratense.*

In Table V the results of attempts to build up *Puccinia graminis phleipratensis* from various sources on barley and oats are given. It is possible to maintain the rust for considerable periods of time, both on oats and barley, but the writers have never been able to keep it indefinitely. The number of uredinia became smaller, usually with each successive transfer, and the individual uredinia quite often decreased in size. Eventually so few spores are produced that only one or two leaves can be inoculated and these then fail to become infected.

TABLE VI.—*Results of successive transfers of Puccinia graminis agrostis to barley, oats, and rye*

No.	Original host.	Place of collection.	Result.
1	*Agrostis alba*	St. Paul, Minn.	$B\frac{9}{16}$—$B\frac{8}{13}$—$B\frac{2}{12}$—$B\frac{3}{7}$; 2—$B\frac{0}{3}$
2	Do.	do.	$O\frac{6}{28}$—$O\frac{0}{11}$
3	*Agrostis stolonifera*	do.	$O\frac{4}{22}$—$O\frac{2}{15}$—$O\frac{0}{7}$
4	Do.	do.	$R\frac{2}{25}$—$R\frac{0}{4}$

As seen from Table VI, *P. graminis agrostis* transfers with difficulty to barley, oats, and rye. The uredinia are always few in number and are practically always very small. It was impossible to increase the virulence of this rust by successive transfers to any of those hosts, although not a great number of experiments were made

GENERAL DISCUSSION

From the foregoing results it seems perfectly safe to conclude that if bridging and adaptation do occur, they occur rarely. Although all of the possibilities have not been exhausted it would seem that the experiments have been extensive enough practically to eliminate any idea of the possibility of sudden or even gradual changes in the rust under experimental conditions. Furthermore, as a result of extensive inoculations with biologic forms of *P. graminis* from a large number of hosts from widely separated localities (*21*), it seems that observational evidence corroborates the experimental evidence which the writers have obtained. The biologic forms obtained have remained pure and fixed after having once been isolated. It is true that when experiments are carried on for a short period of time only, there may appear to be distinct differences in the different strains of the same biologic form, but when the experiments are carried over a period of years it becomes quite evident that these differences are often due to experimental conditions. It seems quite probable that plus and minus fluctuations may occur, but that there is always a tendency to return to the normal. These plus and minus fluctuations may be induced by host influence or by environmental influence, but with an obligate parasite like *P. graminis* which can not be grown on a standardized medium but must be grown on living plants they are to be expected and the limits must be determined by extensive work.

Barley, which Freeman and Johnson (9) found to increase the range of parasitism of biologic forms has not been found to do this in the writers' experience. Attempts to induce bridging by means of this form have been made continuously for almost four years, and during all of that time no evidence whatever has been obtained that this host is able to change biologic forms. It is true that barley, as well as a number of the grasses, serves as a meeting point for a number of biologic forms and theoretically it seems as though it ought to change their parasitic capabilities. It also appears as though, if all of the common biologic forms of *P. graminis* could be kept on barley for a long period of time, they ought eventually to become practically uniform.

Although it is possible that rusts may change and new biologic forms may develop, it seems more probable that the change is either a very gradual one, extending over long periods of time, or that they change by mutation. No evidence of mutation, however, was obtained in the present investigation. The difference may be one of evolution as compared with experimentally induced change. For practical purposes, however, it seems perfectly safe to say that no certain and marked changes in biologic forms need be expected as a result of growing on bridging hosts; nor does it seem probable that biologic forms are able to gradually adapt themselves to semicongenial hosts by constant association with those hosts. The writers unsuccessfully tried to get evidence of such adaptation. Hybridization may possibly account for some unexplained phenomena and deserves investigation.

It still seems probable that rusts may change as a result of selecting strains from a given biologic form. While there is no positive evidence for this, it seems reasonable that biologic forms may be somewhat analogous to pure lines in genetics and that some forms may possibly be composite from which it is possible to isolate the component pure lines. This last supposition is theoretical only, and is prompted by a study of the *avenae*, *phleipratensis*, and *agrostis* forms. These are similar parasitically and the *avenae* form is variable morphologically, containing spores which after being isolated could be interpreted as being *agrostis* spores and others which might be determined as being *phleipratensis* spores. It is possible, although the experiments do not support the idea strongly, that a number of pure lines might be isolated from the *avenae* strain if extensive attempts were made.

Recently evidence has accumulated which seems to show that some of the apparent bridging obtained by previous investigators may have been due to the fact that several very closely related biologic forms may have been used in the experiments. The discovery of the differential hosts for these biologic forms is largely a matter of accident and the writers are of the opinion that all existing forms of *P. graminis* have not yet been discovered. The fact that several distinct biologic forms which attack various wheats are now known, is especially suggestive. A few varieties of wheat are differential hosts for these forms. Other closely related forms may exist and unless the investigator is lucky in stumbling

onto them, all sorts of puzzling results may be obtained and erroneous conclusions may be drawn.

Biologic forms must have originated in some way and the forces which induced their origin may still be operative but it seems probable that these forces operate so slowly that they do not affect the practical problems of controlling rusts of economic importance. It is highly important, however, that the geographic distribution of biologic forms be ascertained and their relation to the varieties grown or bred for those regions be intensively studied. When this has been done many of the apparently strange and inexplicable phenomena of rapid change in rust resistance will undoubtedly be explained.

SUMMARY

(1) Barberry does not increase the host range of biologic forms; nor does it act as a reinvigorator of the rust. The biologic specialization in the aecial stage is apparently the same as that in the uredinial stage.

(2) Differential hosts must be used to isolate biologic forms from mixtures before conclusive experiments can be made with bridging hosts.

(3) In experiments with a small number of biologic forms and extending over a short period of time there is danger of erroneously concluding that bridging has occurred.

(4) Many hosts equally susceptible to *P. graminis secalis* and *P. graminis tritici* were used as bridging hosts in attempts to change the parasitism of the two forms. Barley, various species of Elymus, Agropyron, Hordeum, and Bromus were used most.

(5) *Puccinia graminis secalis*, which does not attack wheat, but does infect barley readily was cultured on barley and other theoretical bridging hosts continuously for three years during which time more than 2,000 wheat plants were inoculated. The rust acquired no new parasitic capability on account of its association with barley.

(6) *Puccinia graminis tritici* attacks wheat readily, but can attack rye only weakly. Barley is easily attacked. The rust was confined to barley for about 32 months but it never acquired the power of attacking rye more readily than rust taken directly from wheat.

(7) Several species of Elymus, Agropyron, Hordeum, and Bromus were used as bridging hosts for both the *secalis* and *tritici* forms; but no bridging resulted.

(8) Attempts to change the parasitism of *P. graminis avenae* by means of bridging hosts were also unsuccessful.

(9) The taxonomic relationship theory of bridging was tried. If plant C can not be attacked by the rust from a taxonomically distant host plant A, it can not be attacked after the rust has been grown on a form B intermediate taxonomically between A and C.

(10) No one so-called bridging host nor any combination of such hosts enabled any biologic form tried to infect naturally immune plants nor to infect a highly resistant plant more readily.

(11) Many attempts were made to increase the virulence of biologic forms on resistant hosts by successive transfers to these hosts. *P. graminis tritici*, *P. graminis avenae*, *P. graminis phleipratensis*, and *P. graminis agrostis* were used. The results indicated that rust forms do not gradually adapt themselves to resistant or semicongenial hosts.

(12) Biologic forms seem to be roughly analogous to pure lines. Plus and minus flucutations may occur, but there is always a tendency to return to normal.

(13) It is possible but not demonstrated that some biologic forms may be mixtures from which "pure lines" can be isolated. *P. graminis avenae* is a possibility.

(14) The facts given in this paper do not support the conclusions of previous workers that the pathogenicity of biologic forms is easily changed by host influence.

(15) From the practical standpoint the constancy of biologic forms is of great importance. Breeding for rust resistance can proceed with considerable assurance that the same rust will not adapt itself quickly to new varieties.

(16) Biologic forms may have arisen either by mutations or by gradual process of evolution. These processes may be operative yet, but the writers have not been able to detect any mutation nor to induce perceptible evolutionary changes experimentally. The possible rôle of hybridization will be investigated.

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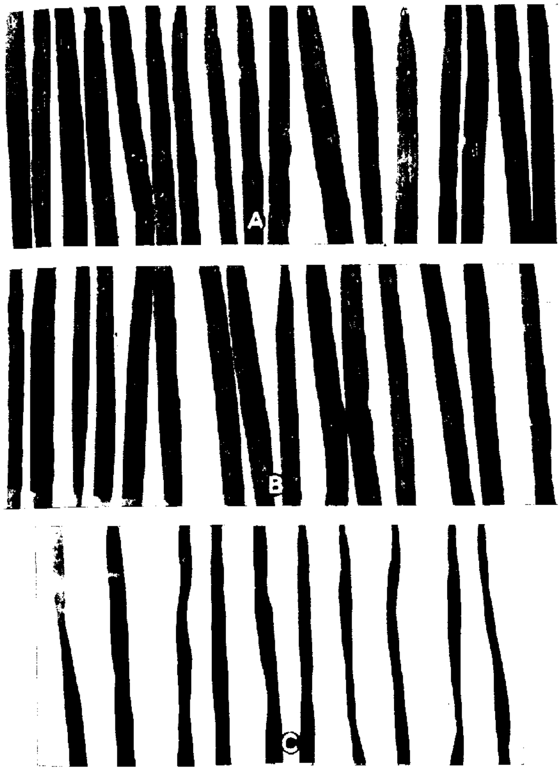
PLATE 17

A, B.—*Puccinia graminis tritici* from *Hordeum jubatum* (Moorhead, Minn.) on rye after having previously spent 2 urediniospore generations on rye, 4 on barley, 1 on rye, 2 on wheat, and 5 more on barley. Twenty-one out of thirty-two blades inoculated became infected; the uredinia produced were very small and surrounded by hypersensitive areas.

C.—*Puccinia graminis tritici* from *Hordeum jubatum*, originally from Moorhead, Minn., but with subsequent history of R_2—B_4—R_1—W_2—B_5—R_1. (See fig. A.): Normal infection on wheat.


A
B
C

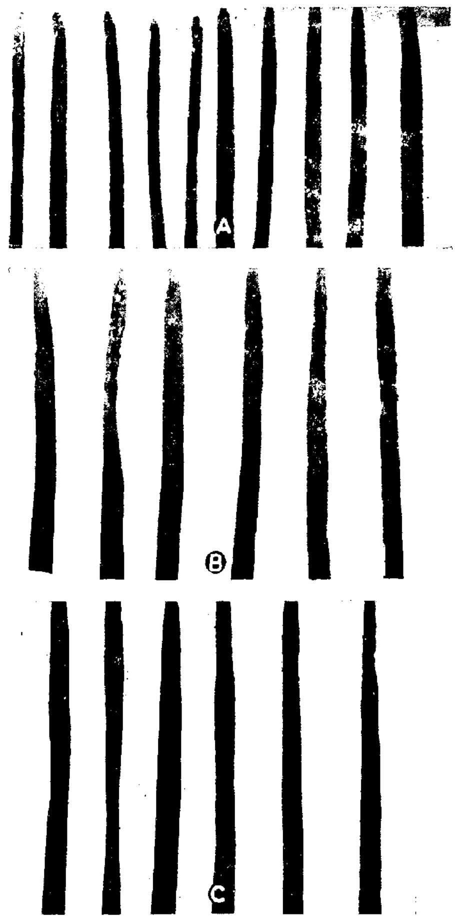


PLATE 18

A.—*Puccinia graminis tritici* from *Hordeum jubatum*, originally from Moorhead, Minn., but with subsequent history of R_2—B_4—R_1—W_2—B_5—R_1: Small uredinia and sharp flecks on rye.

B, C.—*Puccinia graminis tritici* on wheat. Normal development of uredinia produced by inoculating with urediniospores of the following life history: B. *P. graminis tritici* (St. Paul, Minn.) B_{31}—*Agropyron tenerum*$_1$—*A. repens*,—W_1—*A. repens*$_1$. C. *P. graminis tritici*(St. Paul, Minn.) B_{31}—*Agropyron tenerum*$_1$—*A. repens*$_1$—W_1—*A. tenerum*$_1$.

EXPERIMENTS IN FIELD TECHNIC IN PLOT TESTS [1]

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INTRODUCTION

In summarizing the replies to a circular letter sent out to Experiment Stations in the United States and in Canada, one is impressed with the wide variations in the size and shape of the plots and in the width of alley between plots in variety, cultural, rotation, and fertility work. The general size of plots varies from $\frac{1}{120}$ to ⅛ acre and in shape from 3 to 33 feet wide and 36 to 272.25 feet long. The width of alley between plots varies from none to 8 feet. Twenty-two STATIONS report the removal of end borders from plots, and nine report no alleys, with grain removed to form pathway or the removal of one side border drill row from either side of each plot.

This lack of uniformity in experimental technic in plot tests, particularly with regard to alley effect, which involves shape and size of plot, raises several questions, among which are the following:

(1) How far within plots is alley effect operative?

(2) What is the increase in yield due to alley effect?

(3) In plots surrounded by alleys, is the effect of the additional space the same on all varieties?

Data obtained at University Farm in 1917 from which to determine the most desirable methods in plot variety testing and cultural trials form the basis of this paper. Although the results are for one season only, they appear to be sufficiently conclusive to warrant their publication. It is hoped that similar work may be done elsewhere, and the collected data serve as a basis for the adoption of more uniform methods in plot tests.

REVIEW OF LITERATURE

Montgomery (*6*)[3] suggests two methods of obviating competition between the larger and more rapidly growing varieties of wheat and oats grown in 8- and 10-inch rows—namely, to plant only similar varieties in adjacent rows and the use of block plots. The possible effect on the

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[2] The authors wish to acknowledge their appreciation of the assistance given by Mr. R. J. Garber, Assistant in Farm Crops, in compiling the data.

[3] Reference is made by number to "Literature cited," p. 270.

yields of different varieties when grown in plots surrounded by alleys is not considered. Shape seemed to be of little importance in plots not surrounded by alleys.

Barber (*1*) noted that plants in the borders of plots surrounded by alleys were more thrifty as indicated by a greater number of culms per plant, a longer period of growth and a higher yield of grain. Yields obtained at the Maine Agricultural Experiment Station for individual oat plants utilizing 36 square inches of space as compared with others occupying 6 square inches showed that the former yielded four times as much grain as the latter. Additional evidence of the increased yield of plants with greater space for development is quoted from Wacker (*10*).

Based on the investigations of Ten Eyck (*9*) and Rotmistrov (*8*), Barber (*1*) makes the estimate that plants growing in an area 6 inches wide within the borders of plots receive benefit from the adjacent alleys. Tables are given showing that the nearer a plot is to the form of a square, the lower is the percentage a 6-inch strip around the border of a plot to its total area. From this it is concluded that (*1*) shape as well as size of plot must be considered in variety tests, and (*2*) that square plots give more accurate results in variety testing than rectangular plots of the same size.

Mercer and Hall (*5*) conclude that, based on data secured from plots removed from large fields and therefore not surrounded by alleys, there is practically no difference in the variability of yields obtained from oblong and square plots of the same size.

Jardine (*2, 3*) mentions that the practice of removing the outer drill rows of field plots to eliminate alley effect is followed by the Kansas Experiment Station.

Love and Craig (*4*) show that the same varieties of wheat and oats average 36.51 per cent higher in yield when grown in plots $\frac{1}{200}$ acre in size as compared with the yields in rod rows from which the end borders have been removed before harvesting. The shape of the plots is not given. Differences in stands under the two systems are mentioned.

MATERIAL AND METHODS OF EXPERIMENTATION

In the final variety tests of oats, wheat, and barley conducted on University Farm in 1917, each variety was replicated three times, thus making four plots of each. The plots were 8.5 by 132 feet with 16-foot roadways seeded to grass between each two series and 18-inch alleys between each two plots. The 18-inch alleys represented areas not included within the margins of the adjacent plots. Of each variety, then, there were available for the determination of alley effect four plots made up of seventeen 6-inch drill rows and 1/38.82 (approximately $\frac{1}{40}$) of an acre in size. Eleven varieties of oats, five varieties of wheat, and four varieties of barley were included in the tests.

In the spring the plots were sown the full width of the series, which is usually 134 feet. In order to facilitate the removal of end borders accurately at harvest time, shortly after seeding in the spring, two galvanized-iron wires were stretched exactly 132 feet apart across the ends of the plots. The distance between the wires was checked every fifth plot along the series and the wires anchored securely. A few days before harvest time the grain on the ends of all the plots outside of the lines marked by the two wires was cut and discarded.

At harvest time the drill row next to the alley on either side of each plot was removed by hand, bound and tagged separately. These are referred to as outside border rows. The second drill rows on either side of each plot were next removed by hand and bound and tagged separately. These are referred to as inside border rows. The 13 remaining 6-inch drill rows were then harvested with the self-binder. In harvesting the central 13 rows with the binder there probably was somewhat more shattering of the grain than occurred in harvesting the border rows by hand.

The sizes of the different areas from which yields were determined are summarized for convenience.

Number of 6-inch drill rows.	Dimensions of areas.	Part of an acre.
1	6 inches×132 feet	1/660
13	6.5 feet×132 feet	1/50.77
15	7.5 feet×132 feet	1/44
17	8.5 feet×132 feet	1/38.82

The grain from the portions of the plots which were harvested separately were threshed with a small machine and the yields of each computed. The number of pounds of grain threshed from the central 13 rows and from the two inside border rows of each plot were then added, and from this the yields of the plots with one border row removed from either side of each plot were computed. The number of pounds threshed from all parts of each plot were then totaled, and the yields for the plots with no border rows computed.

DISTANCE WITHIN PLOTS AT WHICH PLANTS ARE SUBJECT TO BORDER EFFECT

For the purpose of determining the distance within plots affected by adjacent alleys, the yields of the outside border rows, the inside border rows, and the average of the central 13 rows of four plots of each variety of oats, wheat, and barley are available. These yields are summarized in Table I.

TABLE I.—*Average yield, in bushels per acre, of oats, wheat, and barley harvested from border drill rows spaced 6 inches apart removed from either side of plots 8.5 by 132 feet and from the central 13 rows remaining after the removal of the border rows*

Source.	Oats.		Wheat.		Barley.	
	Number of rows or plots.	Yield per acre.	Number of rows or plots.	Yield per acre.	Number of rows or plots.	Yield per acre.
		Bushels.		*Bushels.*		*Bushels.*
Outside border rows	88	131.97	40	55.00	32	97.73
Inside border rows	88	87.95	40	40.98	32	64.46
Central 13 rows	44	71.37	20	27.45	16	42.87

The actual yield of the outside border rows is for oats 83.5 per cent, for wheat 100.4 per cent, and for barley 123.3 per cent greater than the average for the central 13 rows for the same varieties. For the three crops represented, the outside border rows yielded 102.1 per cent higher than the average for the central 13 rows of the same varieties.

For oats, the inside border rows averaged 23.23 per cent, for barley 50.36 per cent, and for wheat 49.29 per cent higher in yield than the average for the central 13 rows. For the three crops there was an average increase of 41 per cent in the yield of the inside border rows as compared with the average of the central 13 rows of the same plots. The outside and inside border rows on either side of each plot, together averaged 143.1 per cent higher in yield than the average of the central 13 rows in the same plots. Thus, plants growing in the two outside and two inside border rows on either side of each 18-inch alley, kept reasonably free from weeds, appear to have utilized this area in addition to the space allotted to them within the plots nearly as well as though it had been regularly occupied by three drill rows of plants. This suggests that, within certain limits, width of drill row is a negligible factor in seeding as long as other conditions are uniform. The possible effect of the alleys on the plants farther than 12 inches within the margins of the plots was not determined, but further work is in progress with this object in view.

The plants in the outside border rows particularly were still somewhat green when those in the interior rows were mature. The oat and barley varieties were harvested as soon as the plants in the interior of the plots were mature. By the time the wheat plots could be harvested the plants in the border rows appeared as mature as those in the central rows. The weights per bushel of the oats and barley from the central 13 rows averaged 31.60 and 40.60 pounds, respectively, as compared with 30.08 and 39.50 pounds for that from the outside border rows. There was practically no difference in the average weights per bushel of the oats and barley from the inside border rows as compared with that from the central 13 rows. No differences in weight per bushel were found in the wheat harvested from the three areas of the plots.

The results for oats, wheat, and barley indicate that in plots surrounded by alleys the effect of the additional space extends to the plants occupying areas 12 inches within the margins of the plots and possibly farther. The higher yield of the plants in the outside border rows appears to be due to better nutrition, as is indicated by later maturity.

EXTENT OF INCREASE IN THE YIELD OF PLOTS DUE TO ALLEY EFFECT

From the foregoing it is obvious that the extent of the increase in the yields of plots surrounded by alleys depends upon the relative proportion of the area of the border strip occupied by plants subject to the effect of the additional space to the total area of the plot. In Table II are summarized the percentages (1) of the total areas of plots of different sizes but of the same shape and (2) of plots of the same size, but varying in shape contained in borders 12 inches wide (a) on two sides of plots only and (b) on the ends and sides of plots.

TABLE II.—*Relation of a 1-foot border within a plot to its total area*

Approximate size of plot (fraction of acre).	Dimensions of plot in feet.	Approximate shape of plot.	Area of plot.	Area [a] of border, 1 foot wide on sides of plots only.	Percentage of total area of plot in border, 1 foot wide on sides of plot only.	Area of borders 1 foot wide on ends and sides of plots.	Percentage of total area of plot in border, 1 foot wide on ends and sides of plot.
	Feet.		*Sq. ft.*	*Sq. ft.*		*Sq. ft.*	
1/440	3×33	1:11	99.0	64	64.65	68	68.69
1/220	3×66	1:22	198.0	130	65.66	134	67.68
1/220	6×33	1:5.5	198.0	64	32.32	74	37.37
1/110	3×132	1:44	396.0	262	66.16	266	67.17
1/110	6×66	1:11	396.0	130	32.83	140	35.35
1/110	12×33	1:2.75	396.0	64	16.16	86	21.72
1/55	24×33	1:1.375	792.0	64	8.08	110	13.89
1/160	8.5×33	1:4	280.5	64	22.82	79	28.16
1/80	8.5×66	1:8	561.0	130	23.17	145	25.85
1/80	17.0×33	1:2	561.0	64	11.41	96	17.11
1/40	8.5×132	1:16	1,122.0	262	18.89	277	24.69
1/40	17×66	1:4	1,122.0	130	11.59	162	14.44
1/40	34×33	1:1	1,122.0	64	5.70	130	11.59
1/20	17×132	1:8	2,244.0	262	11.68	295	13.15
1/20	34×66	1:2	2,244.0	130	5.79	196	8.73
1/10	34×132	1:4	4,488.0	262	5.84	328	7.31
1/10	68×66	1:1	4,488.0	130	2.90	264	5.88

[a] In computing the area of a strip 12 inches on the sides of a plot only, 2 square feet, which belong to the end borders, must be deducted from the total area in the two sides strips.

An examination of the percentages given in Table II shows that the relation of the areas affected by the additional space afforded by alleys to the total area of plots is dependent upon both the shape and the size of the plots.

Plots $\frac{1}{440}$ and $\frac{1}{110}$ acre in size and each having a width to length ratios of 1 to 11 have in ends and sides 68.69 and 35.35 per cent, respectively, and in the sides only 64.65 and 32.83 per cent, respectively, of their total area in a 12-inch strip within their margins. Likewise, plots $\frac{1}{40}$ acre in size with a ratio of 1 to 4 for width to length have only approximately half as great areas in 12-inch strips within their margins as those $\frac{1}{160}$ acre in size and of the same shape.

Increased size then in plots of the same shape reduces the area exposed to alley effect. Increase in size of plots where shapes are widely different may not bring about a reduction of the area in the marginal strip exposed to alley effect. As an example of this, plots $\frac{1}{55}$ acre in size and 24 by 33 feet compared with those $\frac{1}{40}$ acre in size and 17 by 66 feet have, respectively, 13.89 and 14.44 per cent of their total area in a 12-inch marginal strip.

As shown in Table II, plots $\frac{1}{220}$ acre in size but having width to length ratios of 1 to 22 and 1 to 5.5 have 67.68 and 37.37 per cent, respectively, of their total area in 12-inch marginal strips. Plots $\frac{1}{40}$ acre in size with the width to length ratios of 1 to 16 and 1 to 1 (approximate) have 24.69 and 11.59 per cent, respectively, of their areas in 12-inch marginal strips. The nearer plots approach the form of a square, the lower the percentage of their total area is exposed to border effect (*1*).

A consideration of the percentages given in Table II brings out the fact that yields from the same varieties or treatments obtained under like conditions, but from different-sized plots, surrounded by alleys are not comparable.

If end and side border effect are similar, it should also be noted in this connection that the removal of end borders only from plots of several different sizes surrounded by alleys may render yields from them still less comparable. A single example using the data included in Table II for plots $\frac{1}{40}$ acre in size, but the width to length approximately 1 to 16 and 1 to 1, respectively, will suffice to make this point clear. With no borders removed the percentage of a 12-inch strip within the margins of the plots to the total area of each are 24.69 and 11.59, and with end borders removed, 18.89 and 5.70, respectively. With no borders removed the percentage of the plots 8.5 by 132 feet exposed to alley effect is approximately twice as great as for those 34 by 32 feet. When the end borders are removed, these percentages are 18.89 and 5.70, respectively, the one being approximately three times as great as the other.

In Table III are given the yields of oats, wheat, and barley grown in plots of the same length, but varying in width. The yields for each crop from the $\frac{1}{110}$-acre plots are considerably higher than those from the $\frac{1}{40}$-acre plots and the yields from the $\frac{1}{40}$-acre plots are somewhat higher than those from the $\frac{1}{10}$-acre plots.

TABLE III.—*Average yields of Ligowa oats (Minnesota 281); Haynes Bluestem wheat (Minnesota 169), and Manchuria barley (Minnesota 105), grown in plots of the same length, but varying in width and border rows removed*

Number of plots.	Approximate size of plots (fraction of acre).	Dimensions of plots.	Crop yield (bushels per acre).		
			Oats.	Wheat.	Barley.
		Feet.			
20........	1/110	3×132	101. 58	42. 49	54. 50
20........	1/40	8. 5×132	83. 70	27. 42	40. 70
20........	1/10	34×132	79. 59	26. 92	....

From the data given it is evident that shape as well as size of plot is of prime importance in considering increases in the yields of crops grown in plots surrounded by alleys. The larger the plots, provided the ratio of width to length remain approximately the same, and the nearer they approach the form of a square the smaller the percentage of total area exposed to alley effect and the less the increase in yield due to this cause.

However, comparatively long and narrow plots can be more easily sown with farm drills and harvested with binders than square plots of the same size. The removal of a sufficient number of border rows from the margins of long, narrow plots to obviate alley effect would appear to be the proper procedure. Leaving no alleys between plots at seeding time and removing a sufficient number of rows from each variety after full heading to provide a pathway of the desired width would accomplish similar results.

In Table IV are summarized the average yields, in bushels per acre, together with the standard deviations for four $\frac{1}{40}$-acre plots (approximate) of each of 11 varieties of oats, 5 varieties of wheat, and 4 varieties of barley (a) with no border rows removed, (b) with one border row on either side of each plot removed, and (c) with two border rows on either side of each plot removed. The yields included in this table are for only one season and are not intended as a variety test report. They are used only in so far as they supply data for the consideration of border effect in variety trials for one season. Comparing the average yields from the four $\frac{1}{40}$-acre plots of each variety, it is significant that in each instance the yields with no border rows removed are the highest, with one border row removed the next highest, and with two border rows removed, the lowest. Considering the average yields for all varieties, the oats, with no border rows removed, yielded 9.14 bushels; the wheat, 5.28 bushels; and the barley, 8.48 bushels per acre more than where two border rows were removed from either side of each plot. When only one border row was removed from either side of each plot, the oat varieties yielded 2.20 bushels, the wheat 1.99 bushels, and the barley 2.86 bushels higher than when two border rows were removed.

TABLE IV.—*Comparison of average yield, in bushels per acre, for four one-fortieth-acre plots (approximate size) with no border rows removed, with one border row removed from either side of each plot, and with two border rows removed from either side of each plot for 11 varieties of oats, 5 varieties of wheat, and 4 varieties of barley*

Crop and variety.	Descriptive note (time of maturity or type).	No border rows removed.			One border row removed.			Two border rows removed.		
		Yield (bushels per acre).	Rank.	Standard deviation.	Yield (bushels per acre).	Rank.	Standard deviation.	Yield (bushels per acre).	Rank.	Standard deviation.
OATS.										
Victory	Medium	99.42	1	4.69±1.12	88.90	1	4.80±1.15	88.57	1	3.50±0.83
Minota [a]	Medium early	87.11	2	2.47±0.59	77.00	2	1.54±0.37	77.06	2	1.13±0.27
Silver mine	Medium	85.68	3	3.45±0.82	78.00	3	2.91±0.69	75.64	3	3.20±0.76
Banner	Medium	82.16	4	5.89±1.40	74.46	4	5.96±1.42	72.82	5	5.90±1.41
Lincoln	Medium	80.71	5	5.65±1.35	72.77	7	5.14±1.23	70.01	7	5.37±1.28
O. A. C. 72	Medium	80.53	6	2.17±0.52	73.87	6	2.38±0.57	71.99	6	2.77±0.66
Iowa 103	Early	79.47	7	4.46±1.06	74.19	5	3.51±0.84	73.66	4	2.99±0.71
Swedish Select	Medium	75.80	8	4.51±1.08	69.23	8	3.46±0.83	66.55	8	3.84±0.92
Kherson	Early	73.40	9	1.59±0.44	67.47	9	1.50±0.41	65.10	9	2.48±0.68
White Tartar	Late	70.97	10	2.49±0.60	65.49	11	1.56±0.37	62.00	11	1.59±0.38
O. A. C. 3	Early	70.40	11	4.64±1.11	65.90	10	4.89±1.17	64.33	10	5.34±0.27
Average		80.51		3.82±	73.57		3.42±	71.37		3.47±
WHEAT.										
Marquis	*vulgare*	37.30	1	.58±1.39	33.41	1	.65±0.15	31.69	1	.31±0.07
Preston	*vulgare*	36.49	2	2.78±0.66	31.58	2	1.80±0.43	29.51	2	1.38±0.33
Glyndon	*vulgare*	30.82	3	3.55±0.85	27.43	3	3.70±0.88	25.74	4	3.47±0.83
Arnautka	Durum	29.59	4	2.70±0.64	27.60	4	2.05±0.50	25.94	3	2.27±0.54
Acme	Durum	28.44	5	3.59±0.86	26.20	5	3.09±0.74	24.39	5	3.01±0.72
Average		32.53		2.64±	29.24		2.46±	27.25		2.09±
BARLEY.										
Manchuria	Six-row	63.99	1	.59±0.14	47.60	2	.65±0.15	43.89	2	1.37±0.33
Chevalier	Two-row	52.92	2	1.50±0.36	48.15	1	1.77±0.42	46.33	1	1.31±0.31
Manchuria × Manchuria	Six-row	51.85	3	1.68±0.40	45.60	3	.69±0.16	42.81	3	.14±0.03
Manchuria × Manchuria	Six-row	47.72	4	.44±0.11	41.64	4	.23±0.05	38.45	4	.66±0.16
Average		51.37		1.05±	45.75		.84±	42.89		.87±

[a] Minota is a selection made at the Minnesota Experiment Station.

As shown in Table IV, the average standard deviations for the yields of the different varieties of oats, wheat, and barley in the tests with no border rows removed are somewhat greater in each instance than those for the tests with two border rows removed. This suggests the possibility that the probable error for a single determination of yields secured from a single variety grown in plots of a given size may in some instances be greater than the probable error of yields of the same plots after the removal of marginal areas 12 inches in width.

From the data submitted it seems fair to conclude that the extent of the increase in yield of wheat, oats, and barley grown in plots surrounded by alleys depends upon the size and shape of the plots and that the removal of the plants occupying an area at least 12 inches wide within the margins of plots removes alley effect and makes yields more nearly comparable.

ARE ALL VARIETIES AFFECTED ALIKE BY SURROUNDING ALLEYS?

If, when grown in plots surrounded by alleys, the yields of all varieties or of all cultural or fertilizer treatments are increased approximately alike, then, so far as the comparative results of the different varieties in any single test are concerned, the inclusion of the border rows of plots at harvest is not objectionable except in so far as has been noted. An opinion regarding this has been expressed by Barber (*1, p. 82*), as follows:

> All in all, as long as conditions are similar for all varieties of grain in trial in a field of fairly uniform soil, the results of plot tests will show the relative yields of the varieties.

Referring again to Table IV, it will be noted that in the column headed "No border rows removed" the varieties of oats, wheat, and barley are each listed in order of yield, in bushels per acre, for that method of test. Do the varieties maintain approximately the same relation with regards to yields when one and two border rows, respectively, are removed from either side of each plot?

An inspection of the rank of the different varieties, when one and two border rows, respectively, have been removed from either side of each plot shows some changes. If these changes in rank, due to the removal of border rows are fairly consistent throughout the 3- or 5-year period in tests conducted by the three methods, what effect will this have on the final interpretation of results?

The standard deviation for 20 control oat plots is 3.24 ± 0.35 bushels. On using this standard deviation in the formula

$$\frac{\text{Standard deviation} \times 0.6745}{\sqrt{n}}$$

in which n denotes the number of plots, to derive the probable error in bushels per acre for the yields obtained from plots replicated three times (four plots of each variety), the result is found to be 1.09 bushels (*11*). A difference of 4.07 between two results is necessary for odds of 40 to 1 against such a difference in one direction only being due to normal variation (*11*). Multiplying 4.07 by 1.09 bushels gives 4.44 bushels, which is the least difference between any two varieties, which is significant. This figure may be used in a broad way in considering the results for three methods of test. In the test with no border rows removed, Victory yielded 12.31 bushels more than any other variety. Since this difference is greater than 4.44 bushels, Victory oats may be considered the highest yielder under that method of test. Considering the other varieties in a similar way, Minota is not better than Silvermine, but is a higher yielder than Banner or any other variety in the trial yielding less than Banner. Silvermine is not better than Banner, but is superior to Lincoln or any other variety yielding less than Lincoln. Banner, Lincoln, O. A. C. 72 and Iowa 103 are higher yielders than Swedish Select, Kherson, White Tartar, and O. A. C. 3.

The differences in yield per acre for the various oat varieties brought about by the removal of one border row do not necessitate any material changes in the rank of the varieties as discussed for the test made without the removal of border rows.

By using 4.44 bushels as the least significant difference between any two varieties in the test of the oat varieties with two border rows removed, Victory still maintains the lead, but with less margin than in the test with no border rows removed. Minota can not be considered a higher yielder than Silvermine, Iowa 103, or Banner, but is superior to O. A. C. 72 and the other varieties lower than it in yield.

In the test with two border rows removed the variety Iowa 103 shows up as very promising, while in the test with no border rows removed it is of indifferent value.

The standard deviation for 20 control wheat plots is 2.45 ± 0.26. By using the standard deviation in deriving the probable error in bushels per acre for the yields of the wheat varieties as for the oats it is found to be 0.83 bushel. Multiplying 4.07 by 0.83 bushel gives 3.38 bushels as the least significant difference between any two varieties in the tests.

By using the 3.38-bushel difference in considering the varieties in the test with no border rows removed, it is clear that Marquis and Preston are not significantly different in yield, but are superior to the other varieties in the test. The removal of one or two border rows does bring about any significent changes in the rank of the wheat varieties.

The standard deviation for the 20 barley control plots is 2.53 ± 0.27. On deriving the probable error for the barley tests in the same way as the oats, the result is found to be 0.86 bushel. Multiplying 4.07 by 0.86 bushel gives 3.50 bushels as the least difference between any two barley varieties in the tests which may be considered significant.

In the test with no border rows removed the Manchuria cross, listed fourth, is significantly lower in yield than the three other varieties and retains that position in the tests with one and two border rows removed. The rank of Manchuria and Chevalier change in the test with one border row removed, but the difference between their yields is not significant.

In the test of the barley varieties with two border rows removed, the Chevalier yielded 2.44 bushels more than the Manchuria and 3.52 bushels more than the better of the two Manchuria crosses. For this method of test, the Chevalier is not significantly higher in yield than the Manchuria, but may be considered superior to the Manchuria cross. It seems that the barley varieties grown in plots surrounded by alleys are not equally efficient in utilizing the additional space.

The results indicate that, unless there is considerable fluctuation in the response of varieties to border effect when grown in plots surrounded by alleys, in a 3- or 5-year trial, superior types of oats and barley may not be given their true rank unless at least two 6-inch drill rows within the margins of the plots are removed before harvest.

SUMMARY

(1) In plots made up of 6-inch drill rows of oats, wheat, and barley with 18-inch alleys between each two, the outside border rows yielded 83.5, 100.4, and 123.3 per cent, respectively, higher than the average from the central 13 rows. For oats, wheat, and barley the inside border rows (second drill rows within the margins of plots) yielded 23.23, 49.29, and 50.36 per cent, respectively, higher than the averages for the central 13 rows. Alley effect is operative over an area at least 12 inches wide within the margins of plots.

(2) The extent of increase due to alleys varies with the percentage of total area in at least a 12-inch strip within the margin of the plots. This percentage is greater for small plots as compared with larger ones of approximately the same shape and for long, narrow plots as compared with those more nearly approaching the form of a square.

(3) Plots 6 to 8 feet wide and 64 to 132 feet or more in length are more easily sown and harvested with the ordinary farm machinery than plots of the same size, but more nearly approaching the form of a square. Removal of the plants occupying an area at least 12 inches in width in comparatively long, narrow plots apparently obviates the most serious objection to their use in variety test work.

Oats, wheat, and barley, grown in plots 8.5 feet wide and 132 feet long after the end borders had been cut but with no side borders removed, yielded 9.14, 5.28, and 8.48 bushels more, respectively, than when two side border drill rows had been removed before harvest. With only one side border row removed from either side of each plot before harvest, the oat varieties yielded 2.20 bushels, the wheat 1.99 bushels, and the barley 2.86 bushels higher than when two drill rows had been removed.

(4) In a test of oat varieties in plots with two border rows removed, the rank in yield was not the same as when no border rows were discarded. The performance of one variety was very satisfactory by the former and indifferent by the latter method of test.

The indications are that the barley varieties grown in plots surrounded by alleys were not equally efficient in utilizing the additional adjacent space.

The removal of one or two side border drill rows in the wheat varieties did not bring about any significant changes in rank.

CONCLUSIONS

In plots surrounded by alleys plants occupying an area at least 1 foot within the margins are affected by the additional adjacent space.

The indications are that, unless there is a considerable fluctuation in the response of varieties to border effect, when grown in plots surrounded by alleys, superior types may not be given their true rank in tests made in plots from which borders are not removed before harvest.

These results have led to the adoption of the plan of removing the plants from an area at least one foot wide within the margins of variety test plots at the central and substations in Minnesota. These borders are to be removed from the plots between the time of fully heading and harvest.

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